2.3. Target Detection

Given the target segmentation and background statistics, the next step is to detect a region that contains the RNA signal, and for which there is minimal background noise interference. The following target detection algorithm exploits the observation that each print-tip produces a consistent spot morphology:

1. *Target Mask Detection.*
   For a given sub-array which is produced by a single print-tip
   a. for $i = 1 \ldots n$
      i. Calculate the mean gray-level, μ, within the bounding box of the target, T_i,
      ii. Flag the target as a strong target if $\mu > \mu_b + 4\sigma_b$.
      iii. If it is the first target, create an image buffer to store the strong target S;
         Otherwise, find the best matching location between S and T_i via auto-correlation (± 1 only in both directions).
      iv. Add image $S = S + T_i$.
   b. Threshold S to obtain a binary mask M. Threshold value, t, is determined by histogram method [Otsu, 1979], where t is limited by $(\mu_b + 4\sigma_b) \leq t \leq 2\mu_b$. If pixel count within the bounding box is less than 200, the threshold value is $\mu_b + 4\sigma_b$.
   c. Keep largest component in the mask to be the final mask for each print-tip, M.
2. *Target Detection:*
   For the same subarray with pen mask M, and for $i = 1, \ldots, n$
   a. Mask the target T_i by M via autocorrelation method (± 1 only) for both fluorescent channels,
   b. Threshold the target within the masked region. The threshold value is determined by histogram method [Otsu, 1979] but bounded by $(\mu_b + 2.3\sigma_b) \leq t \leq (\mu_b + 4\sigma_b)$, or Mann-Whitney test described in [Chen, 1997].
   c. Union the detected target from both fluorescent channels as M_{T_i}.
   d. Delete the target if the target is too small.

By using the pen-mask, much less noise interaction in the neighborhood of each target is achieved, with some expense of requiring good target segmentation. The program accurately detects the target region, and all other measurements are then taken within the detected region.

References:

[Otsu, 1979] Otsu, N. A threshold selection method from gray-level histogram, *IEEE Trans. Syst. Man. Cyber.* SMC-9(1), 62-66, 1979.

[Chen, 1997] Chen, Y., Dougherty, E. R., and Bittner, M. Ratio-based decisions and the quantitative analysis of cDNA microarray images. *Biomedical Optics* **2**, 364-374 (1997).